REMARKS

Applicants note that the previously filed Amendment in Response to Final Office Action was not entered by the Examiner, and Applicants request that the Amendment not be entered at this time. A complete set of claims and responses to the outstanding issues are contained herein.

Claims 12, 14, 17, 19, 29, and 36-41 are pending. Applicants have cancelled claims 1-11, 13, 15-16, 20-28, and 30-35 and reserve the right to pursue the subject matter in these claims separately. Claims 12, 14, 19, and 36-41 have been amended herein; the amendments do not introduce any new matter to the pending claims.

Rejection of Claims 36 and 37 Under 35 U.S.C. § 112, Second Paragraph

Claims 36 and 37 stand rejected under 35 U.S.C. § 112, second paragraph, as indefinite. According to the Examiner, the indefiniteness is due to insufficient antecedent basis for the term "non-metastatic control" in claim 36 and its dependent claim 37.

Claim 36 as amended no longer recites "non-metastatic control," and the amended term "metastatic control" has antecedent basis. Additionally, Applicants have amended claim 37, as well as claims 19, 39, and 41, to recite "human" instead of "mammal."

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

Rejection of Claims 12, 17, 19, 36, and 37 Under 35 U.S.C. § 112, First Paragraph

Claims 12, 17, 19, 36, and 37 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to meet the written description requirement, because the limitation of "excluding RhoC" allegedly represents new matter. According to the Examiner, this exclusion proviso represents new matter based on Ex parte Grasselli, 231 U.S.P.Q. 393 (Bd. App. 1983), which holds that "mere absence of a positive recitation is not basis for an exclusion." MPEP 2173.05(i).

However, the instant situation does not present a case of "mere absence of a positive recitation," because RhoC is positively recited throughout the application, along with other proteins, such as proteins associated with the actin-based cytoskeleton (e.g., page 9, lines 5-20 of the specification as filed) or the proteins listed in Table 5 as filed. MPEP 2173.05(i) also states: "If alternative elements are positively recited in the specification, they may be explicitly

excluded in the claims. See In re Johnson, 558 F.2d 1008, 1019, 194 USPQ 187, 196 (CCPA 1977) ("[the] specification, having described the whole, necessarily described the part remaining.")." Therefore, RhoC "may be explicitly excluded in the claims." Id. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

Response to the Rejection Maintained in the Advisory Action:

The Examiner maintains that there is no basis identified in the specification for the exclusion of RhoC, because "there is no teaching in the specification of a genus of gene products which alter the actin-based cytoskeleton that excludes RhoC." The Examiner further finds that the instant application differs from In re Johnson, because of an alleged lack of "broad disclosure of a large number of working examples definign [sic] the genus and artificial sub-genus . . . [and] only a showing of three possible species within the extrapolted [sic] genus . . . ."

First, Applicants respectfully traverse the Examiner's characterization of the instant application. Referring to the specification, page 31, lines 14-25, and Table 1, the instant application states that FN "is an extracellular glycoprotein that serves as a ligand for the integrin gamily of cell adhesion receptors." The specification further lists regulators of cytoskeleton, such as RhoC, α-actin 1, α-centractin, and α-catenin. In contrast to the Examiner's assertion, these genes are definite species within the genus of gene products that can alter the actin-based cytoskeleton, and higher expression of these gene products in metastatic tissues and cells is also described. See Table 1 (comparing the numbers in columns M1, M2, SM, F1, F2, or F3 with the numbers in columns P and F0).

Second, Applicants respectfully submit that the holding of In re Johnson is not premised upon how many working examples were provided within the genus or sub-genus. Citing previous decisions, Johnson states: " Inventions are constantly made which turn out not to be patentable, and applicants frequently discover during the course of prosecution that only a part of what they invented and originally claimed is patentable." Id. 1018 (internal citations omitted). Further, "[i]t is for the inventor to decide what bounds of protection he will seek[, . . . to] deny appellants the benefit of their grandparent application in this case [for the limited genus] would let form triumph over substance, substantially eliminating the right of an applicant to retreat to an otherwise patentable species merely because he erroneously thought he was first with the genus when he filed." Id. (internal citations omitted).

The instant case is similar to Johnson in substance, although they may differ in form in that the grandparent application in Johnson disclosed a larger number of working examples. The instant application as filed disclosed and specified several regulators of actin-based cytoskeleton including RhoC that were determined to have enhanced expression in metastatic tissues or cells. See Table 1. The enablement or alleged lack of enablement of a large genus is irrelevant to the issue of whether there is support for the specific exclusion of RhoC. In fact, MPEP 2173.05(i) refers to recited "alternative elements," which may be excluded if recited, not to a genus and species. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims 12, 14, 17, 19, 29, 36-41 Under 35 U.S.C. § 112, First Paragraph

Claims 12, 14, 17, 19, 29, 36-41 stand rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. The Examiner has reiterated that the claims as written are extremely broad because the exact nature of the likelihood of development of metastasis; the stage of the mammal's life the development occurs; the extent of species metastasis covered; the development of metastasis from a neoplasm of any tissue in a human; the range of biological samples that can be used in testing; and nature of alteration of the cytoskeleton causing metastasis are not described in such a way as to enable one of skill in the art to make and/or use the invention.

The claims as amended recite a method of predicting the likelihood of developing a metastatic condition in a human, wherein if the level of one or more gene products in a sample as determined in step (a) of the claim is greater than the level of gene product in the non-metastatic control there is an increased likelihood of developing a metastatic condition, or alternatively, wherein the level of one or more gene products in a sample as determined in step (a) of the claim is the same as the level of gene product in the metastatic control there is an increased likelihood of developing a metastatic condition.

The Examiner has cited various prior art to show the state of the prior art and level of predictability in the art. Specifically, the Examiner has cited multiple references that show seemingly contradictory results with respect to the correlation of the expression level of fibronectin with metastasis. However, all of the references (Christensen *et al.*, [Guo] Linlang *et al.*, Takei *et al.*, and Xu *et al.*, as well as previously cited Fleischmann *et al.*) employ immunohistochemical or immunostaining methodology to determine the level of fibronectin

expression in various lesion or tumor samples. The specificity and sensitivity of the immunostaining methodology are hardly comparable to the array technology employed in the examples of the instant invention which can detect mRNAs at much higher sensitivity and specificity (e.g., page 30, lines 5-10 of the specification). Therefore, while the state of the prior art is unclear, Applicants' examples are based on advanced, reliable technology and clearly demonstrate that increased expression of fibronectin is in fact detected in metastases and correlates with an increased likelihood of developing a metastatic condition.

The Examiner has commented on the existence of working examples, stating that the instant invention does not teach how to obtain a biological sample as recited in the claims at issue. Based on the references the Examiner has cited with regard to fibronectin expression, Applicants submit that biological samples such as autopsy samples as used in Christensen *et al.* (which may be a metastatic control), breast cancer specimens as used in Takei *et al.*, serum and laryngeal normal and cancer tissues as used in Xu *et al.*, liver as used in [Guo] Linlang *et al.*, can obtained by well-known methods in the art.

The Examiner has also raised the issue of working examples. In response to Applicants' remarks filed in the previous Amendment, the Examiner also cites van Gronigen *et al.* (Cancer Research, 55: 6237-43 (1995)), the primary reference cited for an obviousness rejection as discussed in greater detail below. The Examiner states that "the teachings of ban [sic] Gronigen *et al.* provide at least as much as the examples in the specification (with regard to the fact that both show differential expression in tumor cells lines of varying metastatic potential)." Applicants respectfully traverse this characterization of the instant invention. In contrast to van Gronigen's limited teachings on differential expression in tumor cell lines, the instant invention provides ample examples of gene products with enhanced expression in metastases, not cell lines in culture (e.g., page 30, lines 20-24 of the specification). The metastases are lesions obtained from a murine metastasis model, and murine models for studying metastasis are well accepted by skilled artisans. See, e.g., An et al., Anticancer Res. 16:627-31 (1996) and Clin. Exp. Metastasis 15:184-95 (1997); Cher, 5R01CA088028-03; Anderson, 5R01CA090291-02, Grant Abstracts, National Cancer Institute. Further, experimental findings that support the instant invention have also been published by the highly acclaimed, peer-review scientific journal Nature (Clark et al., Nature, 406: 532-35 (August 2000)).

Applicants maintain that the instant invention is an assay for analyzing the expression levels of one or more gene products which Applicants have discovered to be statistically significant markers for predicting the likelihood of the development of a metastatic condition. As the Examiner has pointed out through Steeg et al. (U.S. Patent No. 5,049,662), a secondary reference cited in the rejection under 35 U.S.C. § 103(a) (see below), as well as other references discussed above, one of skill in the art would be able to obtain a sample, determine the level of expression of a gene product in the sample, and compare the expression level of a gene product in a sample to the level of expression of a gene product in a control without undue experimentation. The present invention teaches that by looking at the expression levels of specific gene products, e.g., genes which control the actin-based cytoskeleton, wherein if the level of one or more gene products is greater than the level of the gene product(s) in a non-metastatic control, or alternatively, wherein if the level of one or more gene products is the same as the level of the gene product(s) in a metastatic control, there is an increased likelihood of the development of a metastatic condition.

To reiterate, Applicants provide ample teachings throughout the specification wherein the level of one or more identified gene products, e.g., gene products that alter the actin-based cytoskeleton, has been shown to be higher in individuals with a metastatic condition than non-metastatic condition. Applicants have examined one stage of neoplasm progression (the development of metastases) and teach that there is an identifiable difference between neoplasms that metastasize and neoplasms that do not. As such, Applicants teach a correlation between the increased expression of gene products that alter the actin-based cytoskeleton with an increased likelihood of developing a metastatic condition. Thus, Applicants have demonstrated a role for cytoskeletal organization/re-organization in tumor metastasis.

Accordingly, Applicants claim a method and teach what the components of the method are, how the components are measured, and what the result of the method means. Applicants maintain that the specification and the pending claims enable one of skill in the art to practice the invention as currently claimed without undue experimentation. Reconsideration and withdrawal of the rejection are respectfully requested.

Response to the Enablement Rejection in the Advisory Action:

The Examiner maintains the enablement rejection, finding unpredictability in the prior art. With regards to fibronectin, Applicants respectfully submit that the prior art references contradict each other, not because the expression of fibronectin is in fact unpredictable, but because the prior art references did not employ reliable technology to determine the expression.

Applicants have filed a Supplemental Information Disclosure Statement concurrently that lists the references supporting the validity of animal and cell line tumor models. Consideration of these references is respectfully requested.

Rejection of Claims 12, 17, 19, 36, and 37 Under 35 U.S.C. §103(a)

Claims 12, 17, 19, 36, and 37 are rejected under 35 U.S.C. §103(a) as being unpatentable over van Gronigen *et al.* (Cancer Research, 55: 6237-43 (1995)) ("van Gronigen") in view of Steeg *et al.* (U.S. Patent No. 5049662) ("Steeg").

According to the Examiner, it would have been obvious to one of ordinary skill in the art at the time the application was filed to modify the assay of van Gronigen in order to form a diagnostic assay as taught by Steeg because of the demonstrated correlation between elevated gene expression and metastatic potential.

Claims 12 and 36 are independent method claims comprising a step of determining the level of one or more gene products, excluding RhoC, which alter the actin-based cytoskeleton of one or more tumor cells in the human. Claims 17 and 19 are dependent on Claim 12, and Claim 37 depends from Claim 36. As discussed above, the instant invention provides multiple examples of gene products that alter the actin-based cytoskeleton and are determined to have significantly enhanced expression in melanoma metastases using a well-established, widely used murine model, not cell lines in culture (page 31, lines 15-25 of the specification).

In contrast, van Gronigen teaches differential mRNA display in human melanoma cell lines in culture with different metastatic capacities. By comparing levels of mRNAs from highly metastatic human melanoma cell lines with poorly metastatic human melanoma cells lines, van Gronigen has identified 9 mRNAs that are differentially expressed in the cell lines of different metastatic capacities, one of which being 98% homologous to a human mRNA fragment of laminin B2. Fig 2. of van Gronigen represents the only teaching that laminin B2 mRNA is detected in a highly metastatic cell line in culture (page 6239). Van Gronigen does not teach

determining laminin B2 mRNA level in melanoma metastasis lesions. The only gene product that van Gronigen has determined to be expressed in melanoma lesions is melanoma inhibitory activity ("MIA"), which contradicts van Gronigen's own finding that MIA is only detectable in poorly metastasizing cell lines in culture (page 6241, column 2, second paragraph). Therefore, van Gronigen does not teach enhanced laminin B2 expression in melanoma metastases, which, according to van Gronigen, represent different cell growth stages and may present a contradictory result, similar to MIA. That is, there is no reasonable expectation of success provided by van Gronigen in using laminin B2 to predict the likelihood of developing a metastatic condition in a human, because expression data from van Gronigen's cell line experiments may not correlate with expression levels in metastasis lesions.

Steeg does not provide the teaching or suggestion which van Gronigen lacks. To reiterate, Steeg describes the identification of the NM23 gene and its use for predicting metastatic potential in animal experimental model systems and human cancer. Steeg goes on to describe that NM23 RNA levels were greatest in cells and tumors of low metastatic potential, and declined in highly metastatic specimens. Steeg does not disclose any genes other than NM23, correlate any genes other than NM23 with metastasis or teach that any gene other than NM23 could be used to predict the likelihood of developing a metastatic condition. Moreover, there is no teaching in Steeg that NM23 is involved with the actin-based cytoskeleton.

The cited references, alone or in combination, do not teach or suggest the methods of the instant claims, as amended. As such, the claimed invention is not obvious over the prior art. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

Rejection Based on van Gronigen in the Advisory Action:

Applicants respectfully request that the Examiner clarify the statement that "Applicant argues that there is no teaching tha [sic] NM23 is involved with the actin-based cytoskeleton, but this is a necessary property of a laminin gene product . . . ." The following description of NM23 is available according to OMIM (Online Mendelian Inheritance in Man, a database that is a catalog of human genes and genetic disorders authored and edited by Dr. Victor A. McKusick and his colleagues at Johns Hopkins University and elsewhere, and developed for the World Wide Web by NCBI, the National Center for Biotechnology Information):

Steeg et al. [*J. Nat. Cancer Inst.* 80: 200-204] (1988) gave the name NM23 to a gene they cloned from a murine melanoma cell line. Expression of the gene correlated inversely with metastatic potential. NM23 RNA levels are highest in cell lines with low metastatic potential. RNA levels did not correlate with cell sensitivity to host immune responses. The authors therefore hypothesized that expression of this gene may be associated with intrinsic aggressiveness of the line. Several observations suggested that NM23 activity may be correlated with inhibition of the tumor metastatic process. Bevilacqua et al. [*Cancer Res.* 49: 5185-5190] (1989) concluded that NM23 RNA levels are differentially expressed in human breast tumors and that low NM23 RNA levels are associated with histopathologic indications of high metastatic potential. The product of the NM23 gene is a nucleoside diphosphate kinase, which has been designated p19/nm23. Keim et al. [*J. Clin. Invest.* 89: 919-924] (1992) presented evidence that the expression of the gene is related to cell proliferative activity.

Applicants are not aware of any study that shows NM23 as a "necessary property of a laminin gene product" and therefore request clarification by the Examiner.

CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (617) 951-7000.

If any additional fee is due, please charge our **Deposit Account No. 18-1945**, from which the undersigned is authorized to draw under **Order No. WIBL-P01-534**.

Dated: July 12, 2004

Respectfully submitted,

By_____

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